REMARKS

Claims 23-27 have been examined and stand rejected under 35 U.S.C. §§ 112, first paragraph, 102 or 103. Claim 28 has been withdrawn by the Examiner as directed to non-elected subject matter. Applicants expressly reserve the right to file a divisional application directed to the non-elected subject matter. Applicants have amended claim 23 to correct certain typographical/grammatical errors. No new matter has been added by this amendment.

In view of the remarks below, Applicants respectfully request reconsideration and withdrawal of the asserted objections and rejections.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 23-27 have been rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Specifically, the Examiner contends that "neither the specification nor originally presented claims provides support for a process for preparing an agglutination reagent for detecting typhoid comprising preparing a polyclonal-monospecific antibody" (Office Action at page 7).

On page 5, lines 20-26, the specification states "Flagellin gene sequence specific to *Salmonella typhi* is cloned and expressed by recombinant DNA technology. The expressed recombinant protein is purified by affinity chromatography. Hyper immune sera against this recombinant protein is raised in rabbit. Immunoglobulin fraction of hyper immune sera is separated by ammonium sulphate precipitation. The precipitated immunoglobulins are suspended in 50 mM phosphate buffer (pH 7.2), dialysed and protein content determined." From this disclosure, one of ordinary skill would recognize that the inventors had possession of a polyclonal-monospecific antibody. The antibody is polyclonal because it is derived from a rabbit. The antibody is monospecific because it is specific to the Flagellin gene sequence specific to *Salmonella typhi*.

Accordingly, reconsideration and withdrawal is respectfully requested.

Rejection under 35 U.S.C. § 102

Claims 24-26 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Lim *et al.*, "Detection of Group D Samonallae in Blood Culter Broth and of Soluble Antigen by Tube Agglutination Using an O-9 Monoclonal Antibody Latex Conjugate," J. OF CLIN. MICROBIOL., (July 1987) 25(7): 1165-1168 ("Lim"). Applicants respectfully disagree for the reasons discussed below.

Point I. Lim uses non-carboxylated latex particles, not carboxylated latex particles.

Claim 24 is directed to an agglutination reagent for rapid and early detection of typhoid. The reagent comprises 1% carboxylated latex particles coated with antibody specific to *Salmonella typhi*, suspended in storage buffer.

Lim used "[a] 1% suspension of latex particles (diameter, 0.797 μm; Sigma Chemical Co., Ltd. Poole, United Kingdom) was sensitized in 0.1 M glycine-0.9% sodium chloride buffer (pH 8.2) with an equal volume of *Salmonella* O-9 monoclonal immunoglobulin M (IgM) antibodies (7) ..." (Lim at p. 1165, col. 2). Although this passage does not specifically state that the latex particles are carboxylated, the Examiner contends that they are without providing any evidence to support this contention. Instead, the Examiner improperly shifts the burden to the Applicants to prove a negative – why Lim's latex particles are not carboxylated. For this reason, a *prima facie* case of anticipation has not been established.

Notwthistanding this, Applicants submit print-outs from Sigma-Aldrich's website, which is believed to be the Sigma Chemical Co. referenced in Lim because Sigma-Aldrich is the resulting company from the merger of Sigma Chemical Co. and Aldrich Chemical Co. in 1975. The print-out lists all of the latex beads available by Sigma-Aldrich. They include amine modified polystyrene, carboxylate-modified polystyrene, deep blue dyed, sulfate modified polysterene and plain polystyrene beads. First, since Lim does not indicated that the latex beads are modified, the only reasonable assumption is that the beads are plain polystyrene. If they were something else, one would have expected Lim to expressly state how the beads were modified. Second, the only bead available from Sigma-Aldrich that matches the size of the

particles described by Lim is a plain (or noncarboxylated) polystyrene bead. Sigma-Aldrich only offers one latex bead having a mean particular size of 0.8 μm, LB8. This product is not carboxylated. For these reasons, Applicants believe that Lim used LB8.

Since Lim does not teach using a carboxylated bead, it does not teach each and every limitation recited in claim 24. Claims 25 and 26, depend from and further limit claim 24 and also require utilization of a carboxylated bead. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

Point II. Lim's antibodies are not specific to *Salmonella* typhi.

Claim 24 recites that the carboxylated latex particles are coated with antibody specific to *Salmonella typhi*. In contrast, Lim teaches that the non-carboxylated latex particles are coated with *Salmonella* O-9 monoclonal antibody. The O-9 antigen is a somatic antigen and is a lipopolysaccharide in nature. The antibody generated against the O-9 antigen will not only react with *Salomonella typhi*, but will also react with *Salmonella panama*. Thus, Lim does not teach an antibody that is specific to *Salmonella typhi*. Accordingly, reconsideration and withdrawal of the rejection asserted against claim 24-26 is respectfully requested.

Point III. Lim does not use thimerosal in its storage buffer

Claim 26 recites that the storage buffer comprises thimerosal. Lim teaches storing its non-carboxylated latex particles with non-specific antibodies in the follow storage buffer: 0.1 M glycine, 0.9% sodium chloride buffer, 1% bovine serum albumin and 0.02% sodium azide. It does not teach using thimerosal in its storage buffer. Therefore, it does not teach each and every limitation recited in claim 26. Accordingly, reconsideration and withdrawal are respectfully requested.

Rejection under 35 U.S.C. § 103

Claims 23-27 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Nilsson *et al.* "Microparticles for selective protein determination in capillary electrophoresis," ELECTROPHORESIS, (2001) 22: 2384-2390 ("Nilsson") and Salzman *et al.* (WO 01/040280) ("Salzman"), in view of Sukosol *et al.*, "Fusion protein of Solmonella typhi flagellin as antigen for diagnosis of typhoid fever," ASIAN PACIFIC J. OF ALLERGY AND IMMUN., (1994) 12:21-25 ("Sukosol").

A. Recited Invention

The present invention is an agglutination test using latex particles coated with gamma-globulin fraction of polyclonal nonspecific serum prepared against S. typhi specific flagellin gene product. Thus, the results can be observed with the naked-eye, and without the aide of any instrument. Consequently, the recited invention can be used in the field.

To this end, claim 23 recites a process for the preparation of an agglutination reagent for rapid and early detection of typhoid comprising preparing antibody specific to *Salmonella typhi*, preparing latex particles suspension, and coating of the said latex particles with the said antibody. The antibody is a polyclonal-monspecific antibody specific to *Salmonella typhi*. It is prepared by raising a hyper immune sera against a purified protein encoded by a Flagellin gene specific to *Salmonella typhi*. The polyclonal-monospecific antibody fraction from said hyper immune sera

The latex particle suspension is prepared in part by mixing 1% carboxylated latex particles and 40 mM 2-N morpholinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 in a ratio of 1:1, washing twice with 20 mM MES buffer of pH 5.5 thereby forming a washed latex particle. 1-ethyl-3 (3-dimethyl-amino propyl) carbodiimide hydrochloride (EDC) in 20 mM MES buffer of pH 5.5 is added to the washed latex particle.

The washed latex particle is reacted with the polyclonal-monspecific antibody fraction. The reaction is stopped by adding 1M glycvine (pH 11.0). Thereafter, the antibody coated latex particle is washed with 50 nM glyvine (pH 9.5), 0.03% surfactant and 0.05% sodium azide.

Claim 24 is directed to an agglutination reagent for rapid and early detection of typhoid. The reagent comprises a carboxylated latex particle coated with an antibody specific to *Salmonella typhi*. The particles are stored in a storage buffer. Claims 24-27 depend from claim 24 and further define the particle, storage buffer and antibody.

Claims 24-27 directly or indirectly depend from claim 23.

B. Cited References

Nilsson is directed to a system for detecting protein using two different monoclonal antibodies for human chorionic gonadotropin ("hCG") (Nilsson at abstract). There are two different monoclonal antibodies covalently bound to the latex particles (Nilsson at abstract). Once the latex particles are created, Nilsson teaches washing the particles with Tris-BSA and blocking the carboxyl group with Tris-HCL (Nilsson at page 2385). When a test reagent is mixed with hCG containing sample, an immune complex is formed (Nilsson at abstract). The complex is separated from the latex particles using capillary electrophoresis and detected by UV-Vis detection system (Nilsson at abstract). Since Nilsson discusses using an instrument to detect the hCG, the system is confined to the laboratory, and cannot be used in the field. A capillary electrophoresis is different from an agglutination system.

Nilsson does not disclose several of the limitations recited in claim 23. Namely, Nilsson does not disclose: (1) the use of a polyclonal antibody because it discloses using monoclonal antibodies, (2) binding a monospecific antibody to the latex particle because it teaches binding two different antibodies, each having its own specificity, (3) an antibody specific to *Salmonella typhi* flagellin gene product because Nilsson is directed to hCG, (4) washing the polyclonal monospecific coated latex particle with MES because Nilsson teaches washing with

Tris-BSA, and blocking the carboxyl group with Tris-HCl, or (5) an agglutination reagent because Nilsson teaches capillary electrophoresis.

Nilsson also fails to disclose several limitations recited in claims 24-27. Namely, it fails to disclose: (1) the recited antibody, (2) an agglutination reagent, and (3) the storage buffer.

Salzman generally is directed to a polypeptide derived from flagellin polypeptides used to generated an immune response to gram-negative bacteria (Salzman at abstract). Salzman used a portion of *Salmonella muenchen* specific flagellin gene product to prepare an antibody (Salzman at pages 11-21). The gene product is comprised of less than 160 amino acids, which also match the flagellin amino acid sequence of other gram negative bacteria. Salzman used GST as a tag with the gene sequence to make the fusion protein (Salzman at page 31). Antibodies raised against this protein will not only react with clinical sample of *Salmonella muenchen* and other gram-negative bacteria, but will also react with parasitic infections caused by *Schistosoma japonicum*. Therefore, Salzman does not teach a polyclonal antibody, nor a monospecific antibody because the antibody generated according to Salzman's disclosure will not be specific to *Salmonella muechen*.

Salzman also does not disclose several of the limitations recited in the claim. Namely, Salzman does not disclose (1) a monospecific antibody because the antibody raised against Salzman's protein will react with *Salmonella muenchen,* other gram-negative bacteria and parasites such as *Schistosoma japonicum*, (2) using a latex particle, or (3) the recited reagents.

Sukosol used a 900 base pair gene sequence specific to the *Salmonella typhi* flagellin gene to make a fusion protein with a GST tag in a vector for detection of the antibody (IgM) in serum samples of individuals suspected to have typhoid or related infection (Sukosol at page 23, column 3). Sukosol does not disclose using the *Salmonella typhi* flagellin gene product for making an antibody, binding the antibody to a latex particle or using the antibody in an agglutination reagent.

C. Argument

When making a rejection under 35 U.S.C. § 103, the Examiner has the burden of establishing a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, the prior art must be evaluated based on what it, as a whole, teaches to one of ordinary skill in the art. To establish this, each and every claimed element must be taught or made obvious by the applied references. Additionally, there must be some reason to combine the references in a manner that results in the recited invention.

Here, there is no reason to combine the references to result in a latex particle coated with the recited antibody. Furthermore, the references to do teach or suggest the recited blocking and washing steps, or the storage buffer.

Point I. There is no reason to eliminate Nilsson's second antibody

There is no reason why one would reasonably expect Nilsson's invention to work if the second antibody is removed from the latex particle. Assuming that one was motivated to combine the references, one would create a monoclonal antibody raised against Sukosol's 900 base pair gene sequence specific to *Salmonella typhi*. This antibody would be reacted with Nilsson's latex particle. This latex particle also includes a second antibody.

In contrast, claim 23 recites that the latex particle is coated with a polyclonal-monospecific antibody specific to *Salmonella typhi*, not two different antibodies. Likewise, claims 24-27 recite that the latex particle is coated with an antibody, not two different antibodies. Since there is no reason for one to have removed the second antibody from Nilsson's latex particle, the invention is not obvious over the cited references.

Point II. There is no reason to use a polyclonal antibody instead of a monoclonal one.

Furthermore, there is no reason to use a polyclonal antibody instead of a monoclonal antibody. Nilsson and Salzman teach using a monoclonal antibody on its latex particles. Sukosol provides no motiviation to use a polyclonal antibody instead. Since such a reason is not provided, the recited invention is patentable over the cited references.

Point III. There is no reason to substitute Nilsson's blocking and washing steps with the recited ones.

Additionally, the cited references do not teach the recited blocking and washing steps, and there is no reason to substitute Nilsson's washing and blocking steps with the recited washing step. Nilsson teaches blocking the residual activated carboxyl groups with 0.1 M Trus-HCl, pH 8.0 containing 0.2% BSA, and washing the antibody coated latex particles with Tris-BSA. However, claim 23 recites that the reaction between the latex particle coated and the polyclonal-monospecific antibody is stopped with 1M glycine (pH 11.0), and the polyclonal-monospecific antibody coated latex particle is washed with 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05% sodium azide.

There is no reason for one to substitute Nilsson's blocking and washing steps for the ones recited in claim 23. Accordingly, the recited invention is patentable over the cited references.

Point IV. There is no teaching or reason to use the recited storage buffer.

Nilsson teaches storing its coated latex particles in Tris-BSA. The references to not teach the storage buffer recited in claim 24 and further defined in claim 26. Without such a teaching, there cannot be a reason to modify the cited references in a manner that leads one to the recited invention.

Conclusion

Due to the differences discussed above, a combination of the cited references does not result in the recited invention. Additionally, since the references do not teach using a 6X histine tag, the invention is patentable over the cited prior art.

For these reasons, Applicants respectfully request reconsideration and withdrawal of the objections and rejections, allowance of pending claims 23-27, and rejoinder of claim 28.

Respectfully submitted,

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